LIFE CYCLES OF *CARNEOPHALLUS CHOANOPHALLUS* N. SP. AND *C. BASODACTYLOPHALLUS* N. SP. (TREMATODA: MICROPHALLIDAE)[1]

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Abstract

The life histories of two new microphallid trematodes *Carneophallus* **choanophallus** and *Carneophallus* **basodactylophallus**, from South Louisiana are reported. The natural hosts for *C. choanophallus* are: the raccoon, *Procyon lotor* (L) and the black rat, *Rattus rattus* (L) [definitive hosts], the snail, *Lyrodes parvula* Guilding, 1928 (unspined form) [first intermediate host], and the shrimp, *Macrobrachium ohione* Smith, 1874 and *Palaemonetes pugio* Holthuis, 1949 [second intermediate hosts]; for *C. basodactylophallus*: the raccoon [definitive host], the snail *Lyrodes parvula* Guilding, 1828 (spined form) [first intermediate host] and the blue crab, *Callinectes sapidus* Rathbun, 1896 [second intermediate host]. The cercariae of the two species differ primarily in stylet length and experimentally were not capable of cross-infecting the crustacean hosts.

Cercarial penetration, development and growth of the metacercariae of *C. choanophallus* are described. A 15-month geographical and ecological study of infected *M. ohione* in the Mississippi River is presented.

Introduction

Freshwater shrimp, *Macrobrachium ohione* Smith, 1874, from the shallow water of the west bank of the Mississippi River at Ama, Louisiana were collected in July, 1966. The musculature of the abdomen and the cephalothorax of these shrimp was infected with metacercariae of an unidentified microphallid trematode. Further study showed that the metacercariae were larvae of a new species of *Carneophallus* Cable and Kuns, 1951. An intensive search for the other stages of the life-cycle and an ecological study of the infected shrimp led to the finding of high incidences of densely infected *M. ohione* in several areas near the mouth of the Mississippi River. It was subsequently found that black rats, *Rattus rattus* (L), from Fort Jackson, Louisiana and raccoons, *Procyon lotor* (L), from Pass a Loutre, Louisiana were naturally infected with adults of the new species. Further examination of the small animal runways in the marshes and ponds at Pass a Loutre, Louisiana led to the discovery of the snails, *Lyrodes parvula* Guilding, 1896 (unspined form) which harbor the cercariae. The grass shrimp, *Palaemonetes pugio* Holthuis, 1949, was also found to harbor metacercariae in endemic areas.

During studies on the life history of the microphallid from *M. ohione*, metacercariae of another new, very closely related, species of *Carneophallus* were discovered in the blue crab, *Callinectes sapidus* Rathbun, 1896. Adults of this form were also found in raccoons, and the snail, *Lyrodes parvula* Guilding, 1896 (spined form) was found to harbor cercariae which infect the blue crab.

Materials and Methods

Mississippi River shrimp, *Macrobrachium ohione* Smith, 1874 and the brackish water grass shrimp, *Palaemonetes pugio* Holthuis, 1949, were used in studies and experiments with *Carneophallus choanophallus*, while the blue crab, *Callinectes sapidus* Rathbun, 1896, was used for those dealing with *Carneophallus basodactylophallus*. These crustaceans were collected from various natural habitats with baited traps, dip nets and by dredge-

[1] This study was supported in part by NIH grant No. GM-669 and NSF Grants GB-3036 and GB-5235.

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trawling from a small boat. *M. ohione* collected for studies of shrimp size, incidence, and intensity of infection, were collected with ¼-inch-mesh wire traps (baited) attached to the river bank with 10- to 25-foot-long lines at the ruins of the old Sellers Plantation House at Ama, Louisiana. The shrimp were transported to the laboratory for observation and study in aerated minnow buckets. Measurements of total length of the shrimp are in millimeters from the end of the rostrum to the tip of the telson. The total length measurements were grouped into classes of 5-mm increments. Metacercariae were counted by examination of the transparent shrimp body under low power of a dissecting microscope. Shrimp and crabs were maintained in the laboratory in aerated aquaria or in individual containers. They were fed dried dog food (Austin's Baked Dog Food, Sunshine Biscuits, Inc., Long Island, New York) every other day followed by a change of water several hours after each feeding.

Naturally and laboratory infected shrimp and crabs were used to infect suspected definitive hosts. Metacercariae for infection experiments were counted by placing small pieces of cyst-containing tissue under the dissecting microscope. Laboratory definitive hosts were exposed by feeding metacercariae in small pieces of shrimp or crab tissue.

Excystment of metacercariae was done in 0.75 per cent saline with sharp needles; however, the worms usually excysted spontaneously when allowed to stand in saline for several hours or overnight at room temperature.

Natural definitive hosts were collected in the endemic areas by live-trapping along the bank of the river and by shooting by day and by night from a small boat equipped with a spotlight. In the laboratory, small mammals were killed by a sharp blow to the back of the head, larger mammals were killed by etherization. Within 30 minutes of killing or shooting, the mammal's small intestine was taken out and the adult trematodes were removed by slitting and scraping the gut into containers of 0.75 per cent saline. For larger animals, the small intestine was usually cut into 4-inch lengths before slitting. Examination was done in petri dishes under the low power of a dissecting microscope.

Spined and unspined snails of the species *Lyrodes parvula,* which carried the two species of *Carneophallus* cercariae, were collected with the aid of a large tea strainer from fresh to brackish water marshes and ponds where small animal trails could be seen in the marsh grass (*Spartina* spp.), alligator weed (*Alternanthera* spp.) and the water weed (*Cabomba* spp.). Snails were taken to the laboratory and those observed to be shedding cercariae were isolated. Snails used for incidence studies were crushed on glass plates in a drop of 0.75 per cent saline and observed under a dissecting microscope for cercariae. All snails were maintained in the laboratory in aquaria containing vegetation and water with the salinity adjusted to match that of the collecting area. Since snails were collected from areas near the mouth of the Mississippi and Pearl Rivers, the salinity was found to vary from time to time. The snails were induced to shed cercariae by placing them in small containers of water adjusted to the proper salinity and subjecting them to a slight increase in temperature under an ordinary reading light bulb for several hours.

Shrimp or crabs were preadapted to water of the proper salinity and then exposed to cercariae. Injecting the cercariae into the gill chambers was also effective. If shrimp maintained in the laboratory for 15 days or more showed no metacercariae in their transparent bodies under the low power of the dissecting microscope, they were considered uninfected and were used for infection experiments. Staged studies of metacercarial development in *M. ohione* were done in an incubator maintained at 27 C. Crabs from certain localities (Willswood and Osgood Ponds 12 miles west of New Orleans on U.S. Highway 90) were found to be very lightly infected. These crabs were maintained in the laboratory for three weeks before being exposed to cercariae. Laboratory infections with thin cyst walls were then easily distinguished from natural infections.

Eggs were teased from the uteri of adult worms and their development was followed in containers of water which was changed twice weekly.

Both fixed and living material were used to study all stages of the life-cycles in the present study. Neutral red and nile-blue sulfate vital stains were used. Whole mounts

TABLE 1.
Geographical distribution, incidence and density of *Macrobrachium ohione* infected with *Carneophallus choanophallus* metacercariae from four Louisiana Rivers.

Location	Mile[1]	Number of Shrimp	Number of Collections	Incidence (Per cent)	Average Density per Shrimp
MISSISSIPPI RIVER					
Greenville, Miss.	540	47	1	23.3	0.32
Vicksburg, Miss.	435	10	1	30.0	0.60
New Roads, La.	260	93	2	26.8	0.85
Plaquemine, La.	210	202	2	22.7	0.69
Wallace, La.	135	198	4	20.2	0.38
Bonnet Carre Spillway, La.	125	308	3	22.0	0.66
Ama, La.	115	9994	120	22.4	0.84
Empire, La.	30	13	1	46.1	2.69
Fort Jackson, La.	20	661	9	67.6	3.65
Baptiste Collette Pass, La.	10	246	4	64.4	5.28
Pass a Loutre, La.	−5	484	8	71.0	4.23
ATCHAFALAYA RIVER					
Morgan City to Charenton, La.		125	2	0.8	0.008
RED RIVER					
Highway 107 at Vick, La.		25	1	0.0	0.0
WEST PEARL RIVER					
Highway 90 to Pearl River, La.		0	3	0.0	0.0

[1] Mile numbers indicate the distances above and below (−) Head of Passes, La. at the mouth of the Mississippi River (see *Flood Control and Navigation Maps of the Mississippi River, Cairo, Illinois to the Gulf of Mexico*, 34th Ed. (1966) prepared by the U. S. Army Corps of Engineers. 68 maps, 12 charts, 43 sheets).

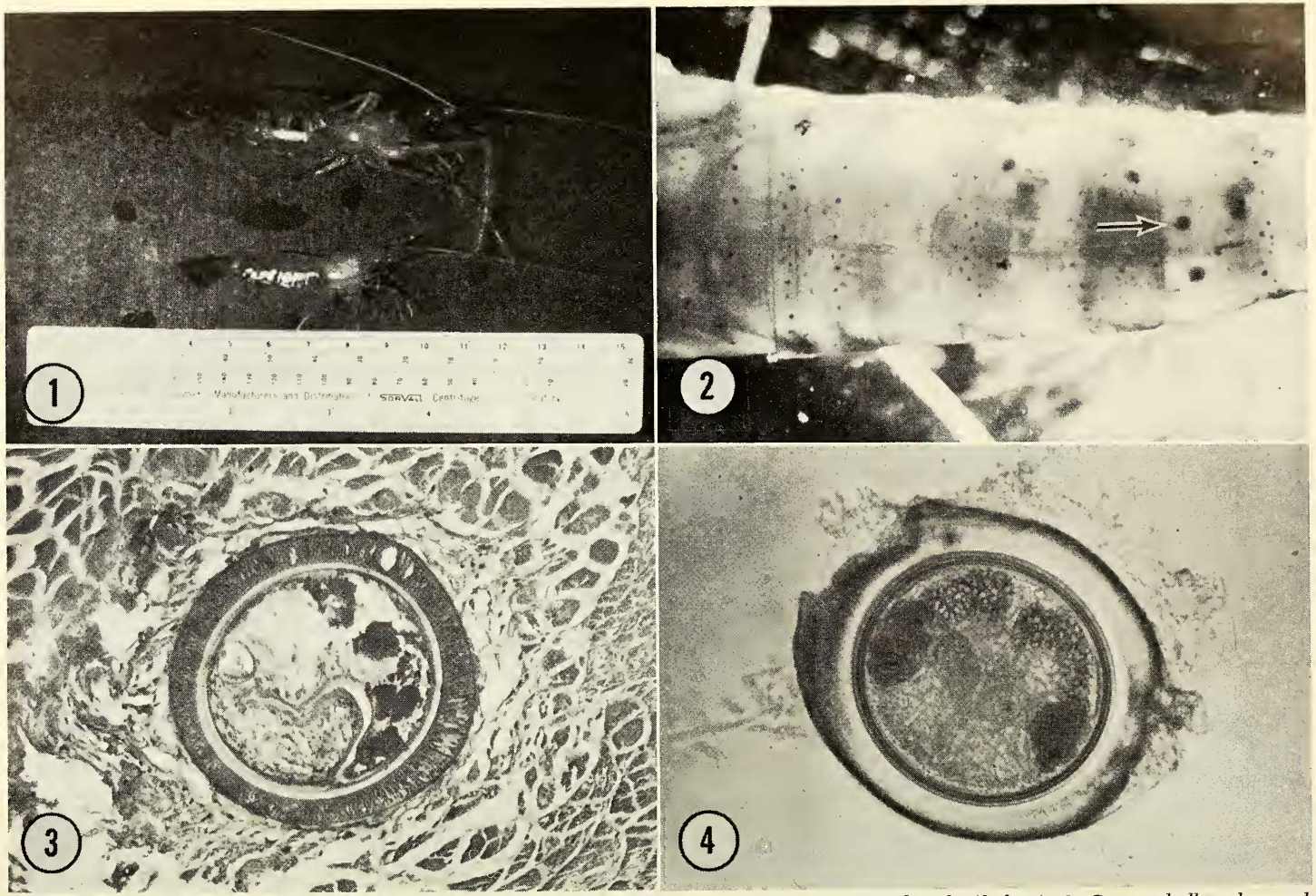
were fixed in alcohol-formalin-acetic acid fixative and stained in Van Cleave's (1953) combination hematoxylin, then mounted in Permount® (Fisher Scientific Company). Adult specimens used for growth studies were killed in hot water before being fixed to assure a standard state of body contraction. Drawings were made free-hand and with the aid of a microprojector. Measurements are in millimeters unless otherwise specified.

Carneophallus from *Macrobrachium ohione* and *Palaemonetes pugio*

The Life-Cycle

In the fall of 1966, Mississippi River shrimp, *Macrobrachium ohione* Smith, 1874, were collected from the shallow water of the west bank of the Mississippi River at Ama, Louisiana. Twenty to 30 per cent of these shrimp had from 1 to 60 microphallid metacercariae in their transparent body musculature (Figs. 1 and 2). Excysted metacercariae revealed a well differentiated preadult trematode which demonstrated all the characteristics of the genus *Carneophallus* Cable and Kuns, 1951. Metacercariae were fed to laboratory rats and mice and ovigerous adults obtained from the intestine 5 to 25 days post-exposure as well as excysted metacercariae were used to identify this trematode as a new species of *Carneophallus*.

An intensive search for the other phases of the life-cycle was made in the Ama, Louisiana region. All snails and suspected definitive hosts taken from this study area were examined for microphallid cercariae and adults with negative results. A geographical and ecological survey of the infected shrimp (Table 1) finally pointed the way to marsh and pond areas between the distributaries at the mouth of the Mississippi River near the Louisiana State Fish and Wild-Life Camp at Pass a Loutre, Louisiana, where the incidence and density of shrimp infection was considerably elevated. Examination of numerous animals observed to be feeding on shrimp revealed that the raccoon, *Procyon lotor* (L), and the black rat, *Rattus rattus* (L), were naturally in-



Figures 1-4. 1. *Macrobrachium ohione* (Mississippi River shrimp) typical female (above) and male (below). 2. *Carneophallus choanophallus* metacercaria (arrow) in abdominal musculature of *M. ohione*. 3. Paraffin section of mature *C. choanophallus* metacercaria in *M. ohione* demonstrating the layers of the cyst wall. 4. Fifty-day-old laboratory infection (room temperature) of *C. choanophallus* metacercaria dissected from *M. ohione* muscle.

fected with species of *Carneophallus,* includ- the new species.

Raccoons and rats were observed to make runways in the alligator weed (*Alternanthera* spp.), marsh grass (*Spartina* spp.) and the water weed (*Cabomba* spp.) in the Pass a Loutre area. With the aid of a large tea strainer, amnicolid snails, the spined and unspined forms of *Lyrodes parvula,* were collected from runways and brought back to the laboratory where they were observed to be infected with microphallid cercariae. Although the cercariae shed from the two snail types appeared to be identical at first, only those from the unspined form of *L. parvula* were capable of infecting shrimp. Careful examination with vital stains later demonstrated morphological differences in the cercariae. Usually the unspined form of *L. parvula* was found on vegetation in the open ponds where *Cabomba* predominates, whereas the spined form of *L. parvula* was found along the edges of the ponds and marshes in the dense vegetation. Young shrimp, *M. ohione,* and *Palaemonetes pugio* in these ponds were heavily infected with the new metacercariae.

While field work was under way, laboratory experimentation was conducted to determine which of several animal types could serve as possible definitive hosts. The results of the survey were as follows: All mammals including white mice (ICR strain), white rats (Sprague-Dawley strain), cats, a raccoon, guinea pigs, meadow mice (*Microtus montanus*) and cotton rats (*Sigmodon hispidus*), which were fed metacercariae yielded good numbers of ovigerous adult worms 12 hr to 25 days post-exposure. Of eight chicks and four Pekin ducklings fed large numbers of metacercariae, only one chick upon necropsy yielded two nonovigerous worms 24 hr post-exposure. The others sacrificed 24 to 96 hr post-exposure yielded no worms. Frogs, *Rana pipiens* and *R. grylio,* maintained at room temperature yielded nonovigerous worms upon necropsy 12 to 96 hr post-exposure. Turtles, *Graptemys* spp., did not become infected. All mammals fed metacercariae from *M. ohione* and *P. pugio* were susceptible to infections producing ovigerous specimens. The frogs, turtles and birds were either not susceptible or unable to produce mature ovigerous infections under normal conditions.

These studies demonstrated that the raccoon and the black rat are natural definitive hosts for *Carneophallus* metacercariae from *M. ohione* and *P. pugio;* however, any shrimp-eating mammal could probably serve as well. The first intermediate host is a snail, the unspined form of *Lyrodes parvula,* which shed cercariae demonstrated to produce experimental infections in shrimp, *M. ohione* and *P. pugio,* identical to those found in nature. This life-cycle of the *Carneophallus* metacercariae from *M. ohione* and *P. pugio* is illustrated in Figure 5 A. The description of this new species follows:

Description of the Stages of the Life History of Carneophallus **choanophallus** n. sp.

The Adult (Figures 6-8)

SPECIFIC DIAGNOSIS: (based on 80 hot water-killed unflattened specimens from *Procyon lotor, Rattus rattus, Rattus norvegicus albinus* (Sprague-Dawley strain), *Mus musculus albinus* (ICR strain) and *Felis domesticus.*) Microphallidae: *Carneophallus.* Body pyriform, sometimes with posterior notch, 0.355 to 0.520 long by 0.200 to 0.340 wide. Forebody 0.155 to 0.325 long. Integument of anterior 1/2 of body spined. Oral sucker subterminal, 0.045 to 0.057 long by 0.050 to 0.062 wide. Prepharynx very short. Pharynx 0.025 long by 0.025 wide. Esophagus extending from pharynx to approximately anterior 1/3 body, 0.065 to 0.137 long. Ceca two, extending posteriorly and obliquely from cecal bifurcation at posterior end of esophagus, 0.037 to 0.160 long. Acetabulum equatorial, mesial, 0.042 to 0.062 long by 0.042 to 0.062 wide. Sucker ratio 1:0.89 to 1.03.

Genital pore sinistral to acetabulum, followed by genital atrium diameter approximately equal to acetabulum. Testes two, side by side in posterior 1/3 body, edges smooth, oval in outline: right testis 0.011 to 0.051 long by 0.027 to 0.079 wide; left testis 0.056 to 0.118 long by 0.037 to 0.071 wide. Vasa deferentia joining anterior to acetabulum, connecting with right posterior margin of seminal vesicle. Seminal vesicle club-shaped, preacetabular, transverse to longitudinal axis of body, tapered to form slender sperm duct surrounded by prostate gland cells at distal tip. Sperm duct connecting with fleshy intra-atrial collared geni-

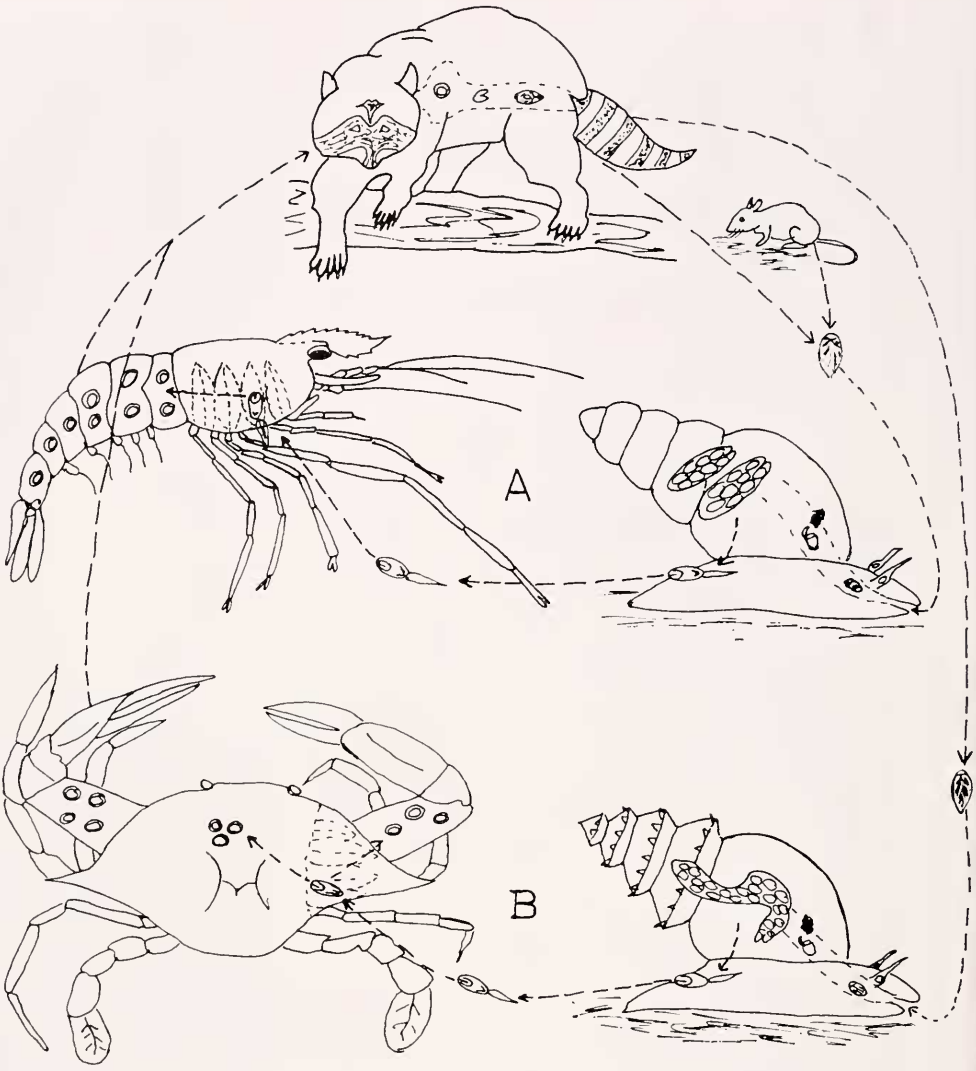


Figure 5. Diagrammatic representation of the life-cycles of (A) *Carneophallus choanophallus* and (B) *Carneophallus basodactylophallus.*

tal papilla, 0.045 to 0.062 long by 0.040 to 0.062 wide. Ovary between seminal vesicle and dextral testis; oval to oblong in outline, edges lightly lobed, 0.030 to 0.077 long by 0.037 to 0.087 wide. Uterus descending from mesial intertesticular Mehlis' gland to fill posterior body, ascending along body wall to posterior tip of dextral cecum, descending to posterior body, ascending along body wall to posterior tip of sinistral cecum, connecting with slightly enlarged metraterm surrounded by gland cells which in turn connects with genital atrium. Vitellaria composed of 6 to 11 coarse follicles on each side of body, extending from posterior ends of ceca to posterior 1/8 of body. Uterine eggs operculate, 16 to 17 microns long by 10 to 12 microns wide. Excretory vesicle V-shaped; main stem extending anteriorly from mesial excretory pore at posterior end of body, forking at level of posterior border of vitellaria, extending to level of testes. Flame cell pattern 2 $[(2+2)+(2+2)]=16$.

HOSTS: *Procyon lotor* (L) [type host] and *Rattus rattus* (L), [natural hosts]; *Mus musculus albinus* (ICR strain), *Rattus norvegicus albinus* (Sprague-Dawley strain), *Felis domesticus, Cavia porcellus, Microtus montanus* and *Sigmodon hispidus,* [laboratory hosts].

LOCATION: Small intestine.

TYPE LOCALITY: Mouth of the Mississippi River at Pass a Loutre, Louisiana.

HOLOTYPE: U. S. Nat. Mus. Helminthological Coll. No. 70428.

Carneophallus choanophallus is distinguished from the other species of the genus by the collared male papilla and the prominent follicles of the vitellaria extending from the tip of two ceca, a position well anterior to the acetabulum, to past the testes well into the posterior region of the body. The very short prepharynx, even in extended living worms, distinguishes it from all types except *C. turgidus* Leigh, 1958.

Growth in Different Hosts

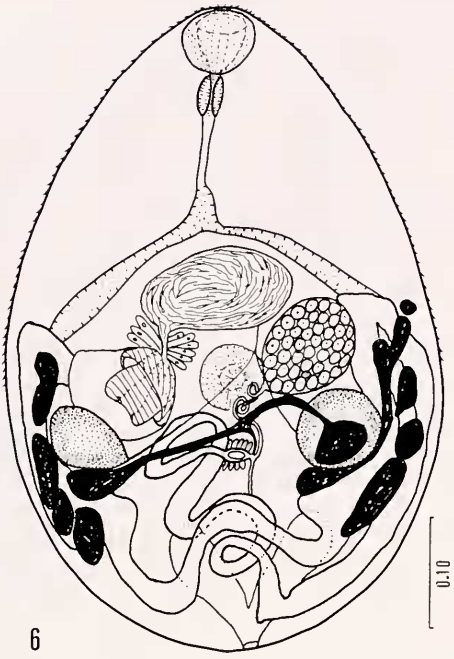
An attempt was made to determine if growth variations occurred within hosts of the same species. Four groups of 6 to 12 laboratory white mice (ICR strain) were fed approximate numbers of *C. choanophallus* metacercariae from natural infections of *M. ohione,* and the mice were sacrificed 48 hr (2 days), 5 days and 25 days post-infection. The worms were removed from the intestine, killed in hot water, fixed in alcohol-formalin-acetic acid, stained and mounted in Permount® for measurement. Computations were based upon measurements of 20 worms for each determination. The comparative similarity of organ sizes is illustrated in Figure 13, in which the body length and width, oral and ventral sucker width and ovary length are compared. The ovary decreased in size as the worm aged.

For intraspecific infections, laboratory white rats (Sprague-Dawley strain) and mice, a laboratory domestic cat, a raccoon (*Procyon lotor*) and a frog (*Rana pipiens*) were used. The raccoon was obtained from the Audubon Park Zoo, where it had been maintained for at least two months. However, it was kept in the laboratory 30 days before use in this experiment at which time it was assumed to be free of *C. choanophallus* infection as preliminary studies with other animal hosts had shown that these short-term infections last no longer than 30 days in the laboratory. The other animals (laboratory reared) were assumed to be free of *C. choanophallus.* The hosts were fed approximate numbers of metacercariae from naturally infected *M. ohione,* and were sacrificed 48 hr post-infection with the exception of the frog. Since *C. choanophallus* does not become ovigerous in frogs maintained at room temperature, a frog was placed in an incubator at 37 C for 15 hr post-exposure after which time it yielded ovigerous adults from the small intestine.

Organs which showed the least amount of variation in dimension were the pharynx and the male copulatory organ. The significant variations in measurements are illustrated in Figure 13. It is seen that for these determinations the adult worms from the white rat and the raccoon were significantly and consistently smaller than those from the mouse and the cat. The causative factors for this difference are not known, but the rat and the raccoon have been found to be the natural definitive hosts. Since the differing dimensions noted are comparatively minor and the genital atrium and male copulatory organ showed no significant variation in dimension or morphology as a result of host variation, the specific diagnosis of *C. choanophallus* is considered to be taxonomically valid.

Longevity of Infection in the Laboratory Mouse

To determine the longevity of *C. choanophallus* infections, 72 mature adult laboratory white mice of the ICR strain (sexes equally divided) were each fed 50 metacercariae from old natural infections of *Palaemonetes pugio.* The mice were divided into 6 groups of 12 mice each and sacrificed at 5-day intervals post-exposure. The small intestine was removed from each mouse, slit open and scraped into saline in a 10.3-cm petri dish for examination under a dissecting microscope. Searching for and counting the worms as they were removed from the petri dish was done 4 times over a 5- to 6-hr period in order to make sure that all worms freed from the intestinal mucosa had fallen to the bottom of the dish where they could be seen. Preceding each examination, the intestine was again scraped and agitated.



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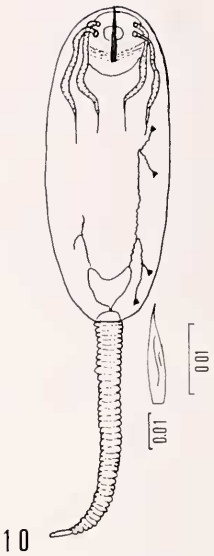

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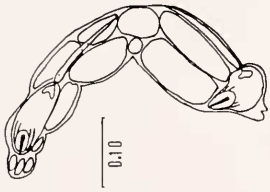

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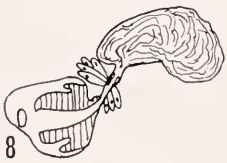

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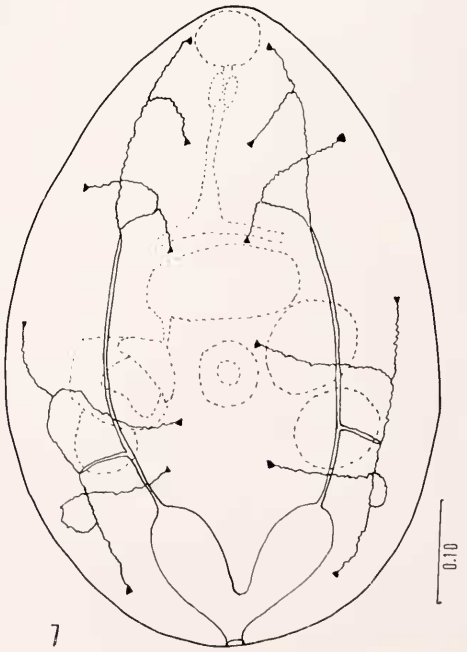

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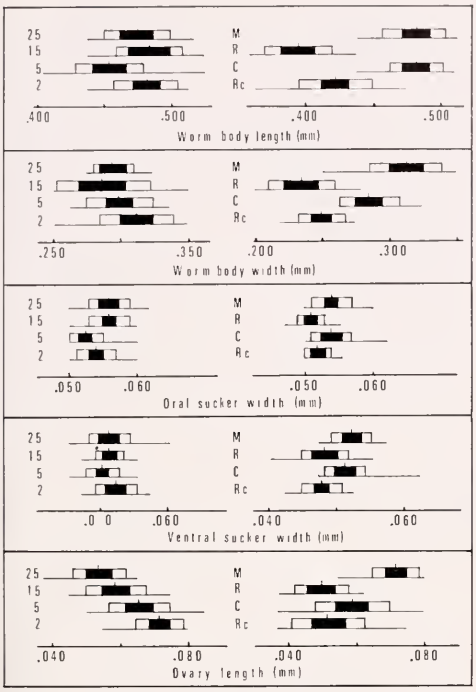

Figure 13. *C. choanophallus* standard length frequency distribution of body length, body width, oral and ventral sucker width, and ovary length (based on 20 hot-water-killed whole mounted specimens per sample). Figures on the left represent 25-, 15-, 5- and 2-day-old infections taken from white mice. Figures on the right represent 48-hr-old infections from white mice (M), white rats (R), a cat (C) and a raccoon (Rc). In each sample the horizontal line indicates the range of the measurements; the cross-bar, the mean; the hollow rectangle, 1 standard deviation on each side of the mean; the solid rectangle, 2 standard errors on each side of the mean.

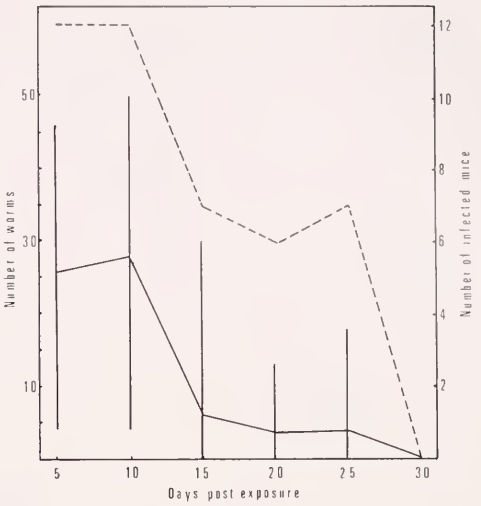

Figure 14. Mean number (_____) and range (vertical lines) of adult *C. choanophallus* from the small intestine, and the number of mice infected (.....) of 6 groups of 12 mice each examined 5 to 30 days post-exposure with 50 metacercariae per mouse.

The results of this experiment are illustrated in Figure 14. On days 5 and 10 post-exposure, all of the mice (24 of 24) were infected with an average of 25 to 28 worms. On the 15-, 20- and 25-day examinations, the number of infected mice was 6 to 7 out of 12, with the average number of worms recovered ranging from 3.5 to 5.8. On day 30, no worms were recovered. Although there were differences between the males and females, these were not considered significant. The general trend was to maintain the infection at a relatively high level for about 10 days, then the density fell to a low level which lasted in some cases up to 25 days but less than 30 days, thus demonstrating the short-term character of infection with *C. choanophallus* in this definitive host. Many of the 15-, 20- and 25-day-old worms were packed with uterine eggs and showed less activity than was observed in the 5- and 10-day infections.

Reinfection of 6 mice 40 days post-exposure resulted in all of the mice becoming infected for a second time, suggesting that previous *C. choanophallus* infections did not make the host refractory to reinfection upon challenge.

The Metacercaria
(Figures 3, 4, and 15-24)

C. choanophallus encysted metacercariae were without exception found embedded in

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Figures 6-12. *Carneophallus choanophallus*. **6**. Adult, dorsal view, drawn from flattened and stained whole mount. **7**. Excretory system, drawn from observations on living excysted metacercariae and adults. **8**. Frontal section of adult genital atrium showing the collared nature of the male papilla. **9**. Egg, taken from the uterus of an adult. **10**. Xiphidiocercaria after emergence from snail. Drawn from living specimen stained with neutral red. **11**. and **12**. Sporocysts from the unspined form of *Lyrodes parvula*.


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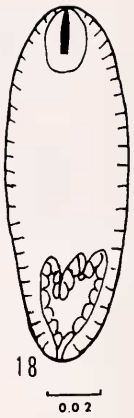
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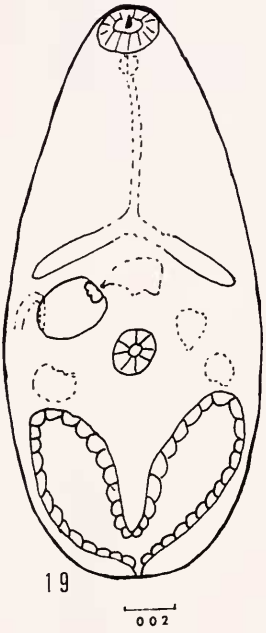
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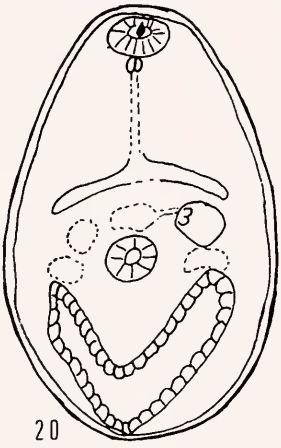
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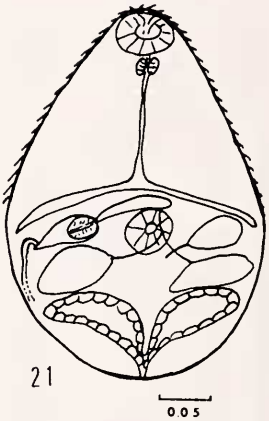
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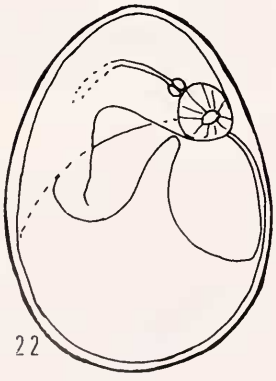
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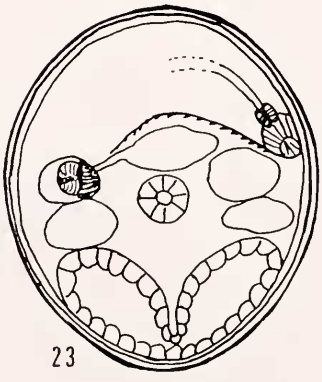
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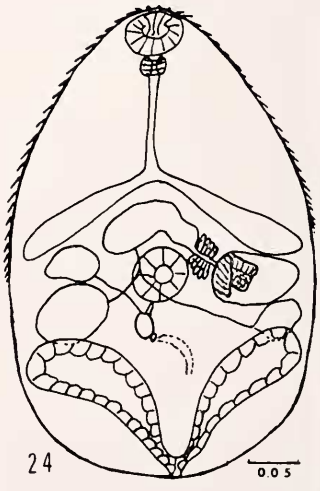
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the shrimp muscle tissue, usually in one or more of the abdominal segments (Fig. 2), occasionally in the cephalothorax and rarely in the appendages.

Infection of the shrimp took place when cercariae from the unspined form of *Lyrodes parvula* entered the gill chamber and penetrated the gill filaments by cutting a slit with the stylet. As the cercaria entered through the slit, its tail became detached and was lost in the current of water passing through the gill chamber. Fourteen shrimp (12 *M. ohione* and 2 *P. pugio*) were observed to be infected by many cercariae in this manner, after which they were placed in an incubator at 27 C.

During the first 18 hr post penetration, the cercariae remained in the gills, and were seen to burrow into the hemocoel. They slowly made their way in the hemocoel to the abdominal musculature where by 24 hr some were found encysted in a very thin and fragile membrane (Fig. 16). The metacercaria, although completely enclosed within this membrane, was seen to be migrating about the hemocoel as it made its way to the abdominal musculature. These newly formed cysts measuring 0.050 by 0.035 in size, grew to 0.090 by 0.040 by 72 hr, and increased in size to 0.095 by 0.050 by 96 hr. At this time, the stylet appeared motile and the oral sucker and excretory vesicle were clearly visible (Fig. 17). Excysted metacercariae of these early stages revealed no additional organs (Figs. 15 and 18). By day 9, the metacercaria contained in its thin (less than 0.002 thick) pliable membrane measured 0.160 to 0.220 long by 0.113 to 0.165 wide (Fig. 20). The cyst wall constantly changed shape as the metacercaria moved about. When the 9-day-old metacercariae were excysted, a remnant of the stylet, the oral and ventral suckers, the pharynx, a small bulbous male papilla surrounded by a thin-walled genital atrium and well-developed excretory vesicles as well as the anlagen of the digestive and reproductive organs appeared visible (Fig. 19). By day 18, the metacercariae measured 0.360 to 0.375 long by 0.240 to 0.245 wide. The cyst wall appeared less pliable and measured 0.002 thick (Fig. 22). All the organs usually seen in metacercariae except the vitellaria and the uterus were observed in the excysted metacercaria although they were reduced in size. Cuticular spines were first observed at this stage. The phallus appeared as a small unlobed muscular bulb within the genital atrium (Fig. 21). On day 23, the encysted metacercariae measured 0.335 to 0.345 long by 0.290 to 0.300 wide. The cyst wall was composed of two membranes, the outer one measured 0.003 and the inner one less than 0.002 thick (Fig. 23). The excysted metacercariae (0.445 to 0.455 long by 0.265 to 0.275 wide) demonstrated clearly the well-developed organs of the preadult metacercaria except for the vitellaria. The collar lobe of the phallus was seen to be developing (Fig. 24).

By day 30, the metacercariae were infective to definitive hosts. At this stage, the excysted metacercariae contained small vitelline follicles and the seminal vesicles were filled with motile sperm. It is suggested that until the vitellaria appear, the metacercariae will not survive in the definitive host, as 4 of 4 attempts to infect mice with young cysts before this stage failed. The encysted metacercariae measured 0.325 to 0.375 long by 0.240 to 0.285 wide. The cyst wall was composed of an inner membrane 0.005 thick and an outer thicker granular wall 0.007 to 0.010 thick. The excysted metacercariae moved about in good condition much as the adults, and measured 0.450 to 0.550 long by 0.305 to 0.320 wide, similar to the adults. In further development, no change was seen in the excysted metacercariae. The size of the encysted metacercariae within the inner membrane apparently reached maximum size about day 30 at 27 C. As time passed, the outer granular layer of the cyst developed into an outer fibrous coat 0.040 to 0.060 thick, external to an extremely tough inner hyaline layer 0.010 to 0.015 thick (Figs. 3 and 4). Encysted metacercariae in *M. ohione* have been main-

←

Figures 15-24. *Carneophallus choanophallus* staged development of metacercaria in *Macrobrachium ohione*. (All figures were drawn from living material.) **15-16.** 24-hr stage in gills and muscle, 15 excysted, 16 encysted. **17-18.** 96-hr stage in muscle, 17 encysted, 18 excysted. **19-20.** 9-day stage, 19 excysted, 20 encysted. **21-22.** 18-day stage, 21 excysted, 22 encysted. **23-24.** 23-day stage, 23 encysted, 24 excysted.

TABLE 2.
Incidence and density of infection of different size classes of *Macrobrachium ohione* by *Carneophallus choanophallus* from September, 1966 to November, 1967.[1]

Shrimp Size Class	Numbers of Shrimp: Number of Metacercariae 0	1–10	11–20	21–30	31–40	41–50	51–60	Incidence of Infection[2]	Incidence of Infection: Class	Incidence of Infection: Total Sample	Frequency of Size Class (%) Total Samp.	Total Number of Cysts
20–24	2	–	–	–	–	–	–	0/2	–	–	0.02	–
25–29	23	1	–	–	–	–	–	1/24	4.16	0.01	0.24	1
30–34	15	2	–	–	–	–	–	2/17	11.76	0.02	0.17	5
35–39	60	11	–	–	–	–	–	11/71	15.49	0.11	0.71	18
40–44	560	138	4	2	–	1	–	145/705	20.56	1.45	7.05	437
45–49	1330	420	20	6	3	2	2	460/1790	25.69	4.60	17.91	1804
50–54	1485	489	23	14	4	1	2	533/2018	26.41	5.33	20.19	2376
55–59	1449	392	21	5	2	–	1	421/1870	22.51	4.21	18.71	1504
60–64	1191	306	18	2	1	–	–	327/1518	21.54	3.27	15.18	1071
65–69	787	184	10	2	3	1	1	201/988	20.34	2.01	9.88	859
70–74	438	96	1	2	–	–	–	99/537	18.43	0.99	5.37	264
75–79	177	33	–	1	–	–	–	34/211	16.11	0.34	2.11	92
80–84	107	5	–	–	–	–	–	5/112	4.46	0.05	1.12	13
85–89	131	–	–	–	–	–	–	0/131	–	–	1.31	–
TOTAL	7755	2080	97	34	13	5	6	2239/9994				8444
% of Total Sample	77.9	20.8	.97	.34	.13	.05	.06	22.4/100.0			Mean No. Cysts per Shrimp	0.84

[1] Collections made from the Mississippi River at Ama, Louisiana.
[2] Number of infected shrimp/number of shrimp in specified class.

tained in the laboratory in good condition for up to six months when the shrimp died.

Excystment of the young metacercariae in the laboratory was easily done by simply pricking the thin pliable cyst membrane and teasing the worm out. By the time the fibrous and hyaline layers developed, considerable time and patience was needed to break the tough membranes mechanically. If the cysts were placed in saline at room temperature for 4 to 5 hr, bacterial and perhaps chemical activity would weaken the walls so that excystment was easily done with a sharp needle. With longer time, the worms were usually able to excyst without mechanical assistance.

Geographical Distribution of Infections in M. ohione

During the summer of 1967, a geographical survey of the *M. ohione* infected with *C. choanophallus* metacercariae was conducted in 4 Louisiana rivers. Shrimp collections were made using traps and by dredge-trawling behind a small outboard motor boat. A summary appears in Table 1. The West Pearl River yielded no *M. ohione,* however, infected *P. pugio* were found in great abundance in the brackish water marshes near its entrance into Lake Borgne. *M. ohione* from the Red River were uninfected. From the Atchafalaya River, 2 collections were made upstream from Morgan City, Louisiana. One shrimp harbored a single metacercaria, therefore, the incidence is considered to be very light. In the Mississippi River, the incidence and intensity of infection was found to be relatively constant from Ama, Louisiana (mile 115) to Greenville, Mississippi (mile 540). The incidence and intensity of infection increased in stations downstream from Empire, Louisiana (mile 30). The increase in the first intermediate host, the unspined form of *Lyrodes parvula,* and the definitive host populations near the mouth of the Mississippi River where great expanses of fresh to brackish water marshes and ponds containing *Cabomba* spp., *Alternanthera* spp. (alligator weed) and *Spartina* spp. (marsh grass) remote from disturbances by humans offer the best explanation for the high incidence and intensity of infection of *M. ohione* with *C. choanophallus* metacercariae in these areas.

Incidence and Density of Infection in M. ohione Over a 15-Month Period at Ama, Louisiana

From September, 1966 to November, 1967, systematic biweekly collections of *M. ohione* were made from the west bank of the Mississippi River near the ruins of the old Sellers Plantation House at Ama, Louisiana. During this 15-month period, a total of 9,994 shrimp were captured. Of these, 2,239 (22.4 per cent) were observed under a dissecting microscope to harbor from 1 to 60 metacercariae. Two thousand eighty (20.8 per cent) shrimp harbored 1 to 10 cysts, leaving only 155 (1.5 per cent) shrimp harboring 11 to 60 cysts. Cysts usually occurred in the transparent abdominal musculature, but were also seen in the muscles of the cephalothorax. The trapped shrimp ranged in size from 20 to 85 from the tip of the rostrum to the tip of the telson. The infected shrimp ranged from 25 to 80 in length. The 50 to 54 size class contained the largest number of shrimp in the sample. This size class also showed the greatest incidence and density of infection. The mean density of infection for the total sample was 0.84 metacercariae per shrimp (Table 2).

Figure 25 shows the per cent of infected shrimp compared with the per cent of the total sample by size classes. It is apparent that the incidence of infection with *C. choanophallus* metacercariae is proportional to the percentage of all shrimp in the size classes for the 15-month period. These curves are typical for any month during the collecting period.

Monthly standard length frequency distributions of the uninfected and infected shrimp (Fig. 26) demonstrate that there are variations in these categories during the 15-month sampling period. Graphic representation of the data (Fig. 27) shows that during the months of January to April, the mean sizes of the uninfected shrimp were less than the infected shrimp. In March and April, overlapping took place, and for the remainder of the study time, the mean size of the uninfected shrimp was greater than the infected shrimp. This disparity was more noticeable during the months of May to August.

Figure 27 shows that during the 15-month collecting period there were two maxima

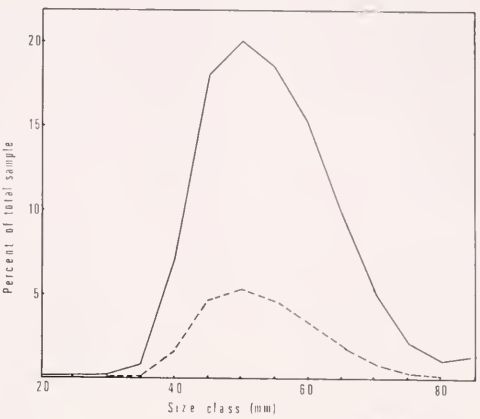


Figure 25. Incidence of infected *M. ohione* (.....) compared with the per cent of the total sample (_____) by size classes for collections made September, 1966 to November, 1967 at Ama, Louisiana.

(November and July) and two minima (January and September) in the mean sizes of the shrimp sample. This suggests two breeding periods. Female shrimp in berry were collected beginning in April and ending in September. As the young shrimp began to appear in the collection samples, the mean size decreased. Growth of the shrimp is indicated by the increase in the mean shrimp size. The greatest growth occurred in the spring.

Figure 28 shows the mean density per shrimp of the total sample compared with the incidence of infection for the collection period. Two maxima (spring and fall) and two minima (winter and summer) are noted with the summer to fall disparity being the greatest. The maximum density and incidence occurred in the early fall. Examination of Figures 27 and 28 together shows that when the sample was of maximum shrimp size (composed of larger shrimp), the density and incidence were minimal. This suggests that recruitment in the shrimp population took place at these times (November to January and June to August), and that these young uninfected shrimp did not appear infected in the collection samples until approximately a month later.

There seem to be two periods of shrimp growth, both of which were accompanied by a decrease in the incidence and density of infection by *C. choanophallus* metacercariae.

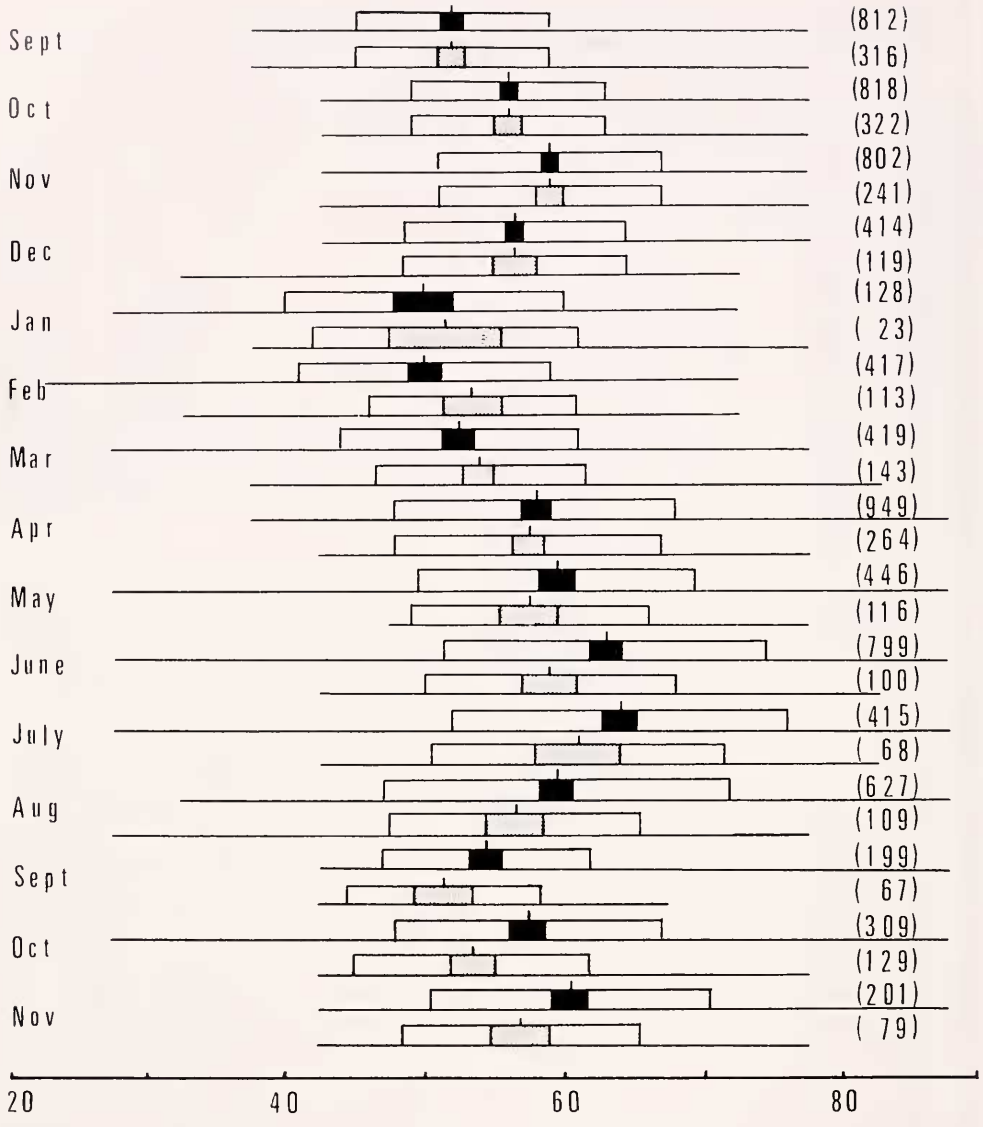
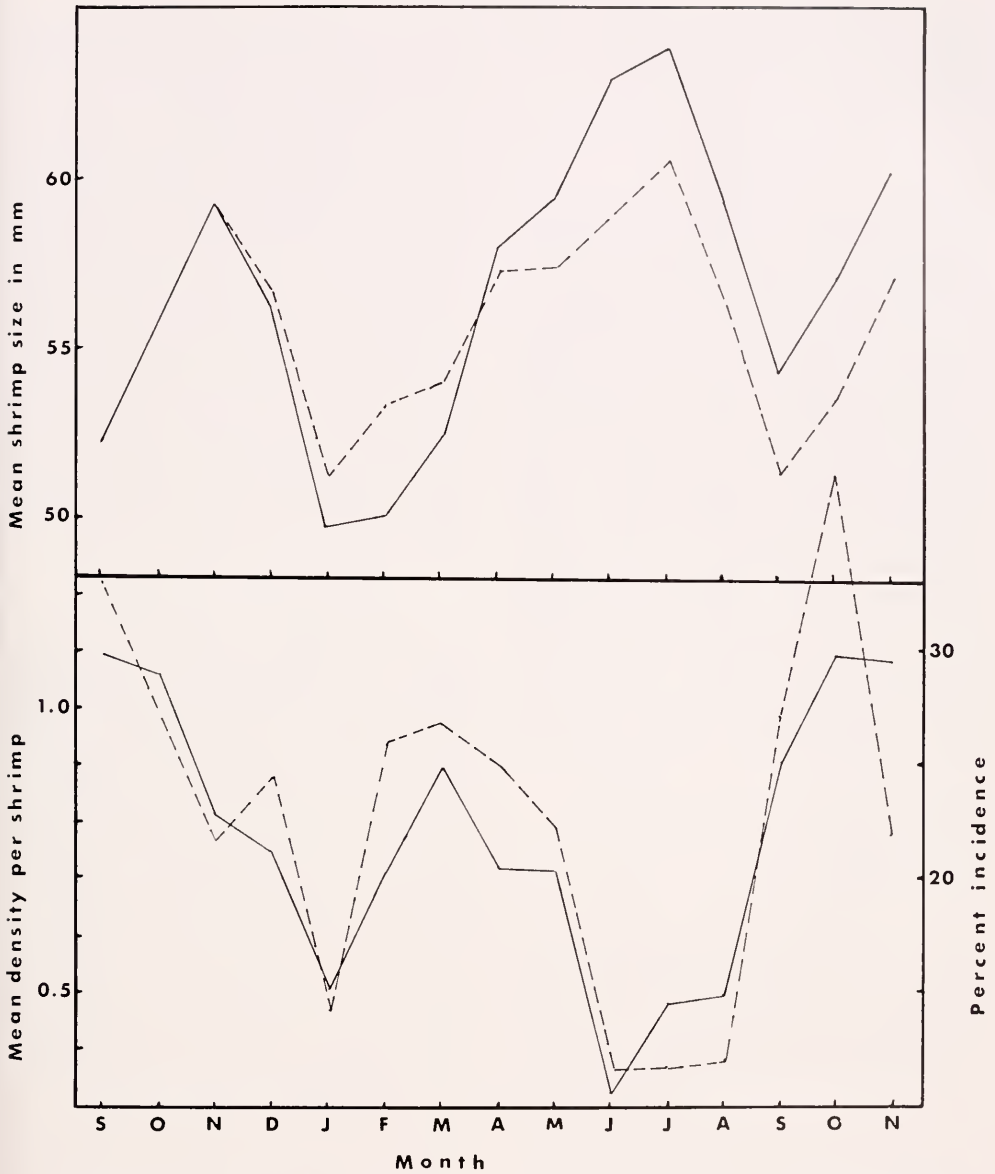


Figure 26. Standard length frequency distribution of uninfected (□■□) and infected (□▨□) samples of *M. ohione* collected at Ama, Louisiana, September, 1966 to November, 1967. In each sample the horizontal line indicates the range of the measurements; the crossbar the mean; the hollow rectangle, 1 standard deviation on each side of the mean; the solid or dotted rectangle, 2 standard errors on each side of the mean. Figures in the parentheses equal the number of shrimp in the sample.

The growth periods are followed by an influx of young shrimp into the sample accompanied by an abrupt rise in the incidence and density of the infected shrimp approximately a month later. This biseasonal increase indicates that most infections probably occurred early in the life-cycle of the shrimp, but it does not preclude the possibility that infection of the shrimp occurred continuously throughout



Figures 27-28. 27. Above. Monthly mean size of the uninfected (_____) and infected (_ _ _ _) *M. ohione*. 28. Below. Monthly incidence (_____) and mean density (_ _ _ _) of the sample of *M. ohione*. Collections were made at Ama, Louisiana September, 1966 to November, 1967.

the year. Observations indicated that the conditions for infection of juvenile shrimp, which live in shallow relatively still water where the unspined form of *Lyrodes parvula* exists, appear to be more likely than those for the adult shrimp, which live in deeper swift moving currents. Laboratory experiments showed that large and small *M. ohione* are both 100 per cent susceptible to *C. choanophallus* cercariae and that superinfection takes place.

The pathological effects of infection on the shrimp are not clear. The larger mean size classes of the uninfected shrimp versus those

of the infected shrimp for June and July suggest that infection interfered with growth, or that mortality was higher in infected shrimp.

The Cercaria
(Figure 10)

Small "monostome" xiphidiocercaria with the characters of the Ubiquita group. The following dimensions were determined using living cercaria, stained with neutral red or Nile-blue sulfate. Body 0.075 to 0.120 long by 0.035 to 0.055 wide, ovoid to elongate. Tail 0.060 to 0.110 long by 0.010 to 0.015 wide at base, with fine cuticular annulations. Oral sucker 0.020 to 0.025 in diameter. Stylet 0.015 to 0.017 long by 0.004 wide, base squared, shaft cylindrical then tapered to slightly ventrally directed point. Digestive system not observed. Two thin pairs of penetration gland cell ducts 0.020 to 0.030 long by 0.003 to 0.005 wide, opening into the anterior portion of the oral sucker lateral to stylet; the lateral gland cell ducts opening posterior to the mesial gland duct apertures. Cystogenous glands not prominently visible. Excretory vesicle U-shaped. Excretory formula 2 [(1 + 1) + (1 + 1)]. Develop in oval or elongate sporocysts containing about 10 to 15 cercariae.

HOST: *Lyrodes parvula* Guilding, 1928 (unspined form).

TYPE LOCALITY: Pass a Loutre, Louisiana.

The cercaria of *C. choanophallus* in general resembled other microphallid larvae but differed from the cercariae of the genus *Microphallus* in the possession of only two pairs of penetration glands and in the molluscan host. The stylet was symmetrical in dorsal aspect but asymmetrical when viewed from the side. The cystogenous gland cells were seen only in cercariae which had emerged from the snail several hours before examination.

Emergence of the cercariae was accomplished by placing the snail in water with the salinity matching that of the field collection site and then slightly elevating the temperature under an electric light bulb. Emergence into water of the incorrect salinity either did not occur, or occurred very lightly and resulted in weakened short-lived cercariae. Normally the cercariae lived up to 48 hr.

Upon emergence, the cercariae swam almost continuously with the posterior part of the body flexed ventrally and the tail lashing vigorously in S-shaped movements. These swimming movements served the purpose of keeping the cercaria suspended in the water off the bottom of the container. If the water was jarred by movement of the container or by touching the surface with a probe, the cercariae stopped swimming for a moment during which time the body would extend and contract once or twice before swimming was resumed. This type of activity aided the cercariae as they were drawn into the gill chamber of the shrimp for penetration of the gills. Contact with the gill filaments stimulated the movements of the stylet as it slashed an opening through which the squirming cercaria penetrated the shrimp.

The Daughter Sporocyst
(Figures 11 and 12)

There are probably two sporocyst generations of *C. choanophallus* in the unspined form of *Lyrodes parvula,* but only the cercaria producing one was observed in the naturally infected snails studied in the laboratory. Out of 200 of the unspined form of *L. parvula* from a locality in which virtually 100 per cent of the *P. pugio* were densely infected, 13 snails (6.5 per cent) were observed to contain *C. choanophallus* cercariae and sporocysts when crushed. The sporocysts were mostly elongate, but some were saccate in shape. They contained 10 to 15 cercariae usually with two of the cercariae ready for release; the others being in various stages of development. The birth pore was terminal as under light cover glass pressure, cercariae were seen to emerge from this position. The opposite end of the sporocyst contained many small rounded structures considered to be developing cercariae and germ balls.

The snails examined contained from 5 to more than 40 sporocysts. Counting beyond 40 was difficult as the sporocysts broke up so that they could not be clearly identified for counting. Since four of the crushed snails were seen to contain more than 200 cercariae, it might be assumed that these snails may have contained more than 40 sporocysts.

The sporocysts measured 0.045 to 0.350 long by 0.020 to 0.080 wide.

The Eggs and Miracidium (Figure 9)

The process of egg formation in the adult worm was observed to be similar to that described by Cable and Hunninen (1940). Three hours post-exposure the adult worms contained a few (1 to 10) eggs in laboratory rats and mice. As seen earlier, it appears that the longer the worm stays in the intestine of the definitive host, the larger the number of eggs found in the uterus.

For egg studies the uteri of 72-hr worms from mice infections were used. Whole worms were cultured in stendor dishes of saline and dechlorinated tap water at room temperature for daily observation. Every other day the water was changed and any debris from the dead worms was removed.

Development of all cultures was as follows: On day 1, the eggs measured 17-18 microns long by 9-11 microns wide. The operculum was clearly visible. Living cells in the process of dividing were observed near either end of the egg. On day 5, the contents of the egg appeared to be divided into sections or clumps. By day 10, a miracidium with the anterior end directed against the operculum was observed. At this stage, it was estimated that 75 per cent of the eggs were viable. By day 15, there seemed to be no change in the appearance of the miracidium. No free swimming miracidia were observed.

Infection of the unspined form of *Lyrodes parvula* by *C. choanophallus* was accomplished by casting whole ovigerous worms, taken from 7 to 20-day mice and rat infections, into aquaria containing the snails. The snails had been collected in a pond at Bonnet Carre Spillway, St. Charles Parish, Louisiana. Two hundred snails were crushed, examined, and judged to be uninfected. Another 200 snails from this collection were exposed and kept in 2 large finger bowls containing dechlorinated water, *Cabomba* spp. and *Alternanthera* spp. Five months later 16 snails survived. Of the survivors, 8 (50 per cent) were infected with *C. choanophallus* sporocysts containing cercariae. It is believed that the high percentage of infection in the surviving snails was the result of the laboratory exposures.

Carneophallus from *Callinectes sapidus*

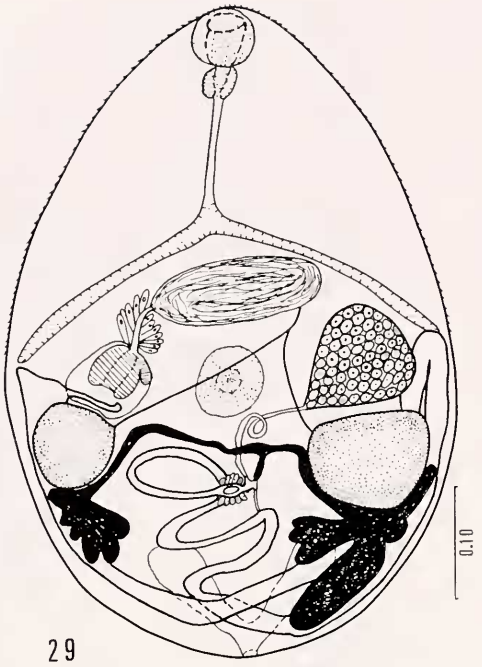
The Life-Cycle

While searching the Pass a Loutre area for the snail harboring the cercaria of *Carneophallus choanophallus* during the summer of 1967, the spined form of *Lyrodes parvula* was found. These snails when brought into the laboratory produced large numbers of a small xiphidiocercaria of the Ubiquita type which fit the suspected description of *Carneophallus* cercariae. The snails also came from a known endemic area. Since many unsuccessful attempts were made to infect *Macrobrachium ohione* with this cercaria, other possible second intermediate crustacean hosts from the Pass a Loutre area were brought into the laboratory for examination. Of these, the blue crabs, *Callinectes sapidus*, for this collection, were found to be 100 per cent infected in the hepatopancreas and muscles with microphallid type metacercariae. When these metacercariae were excysted or fed to rats and mice, the resultant trematodes were found to exhibit the characters of the genus *Carneophallus* Cable and Kuns, 1951. A detailed study of these worms revealed the discovery of a second new species of *Carneophallus*.

As the cercariae from the spined form of *L. parvula* were suspected of infecting the blue crabs from Pass a Loutre, a search was made to find a locality with a population of blue crabs uninfected or very lightly infected with *Carneophallus* spp. of metacercariae. Such a population was located 12 miles west of the Mississippi River bridge on U. S. Highway 90 at the Osgood and Willswood ponds. Ten of these crabs were exposed to cercariae from the spined form of *L. parvula* resulting in medium (50 or more metacercariae) to heavy (100 or more metacercariae) infections whereas the thirty unexposed crabs were either negative or harbored a maximum of three older metacercariae.

In the Pass a Loutre area, all raccoons, *Procyon lotor* (L), examined harbored large numbers of *C. choanophallus* and the second new species. Several birds were also examined but none of these harbored *Carneophallus* spp. No other suspected natural definitive hosts in this area were examined.

The life-cycle is as illustrated in Figure



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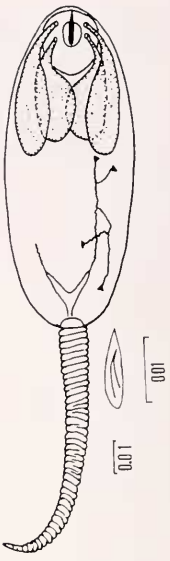

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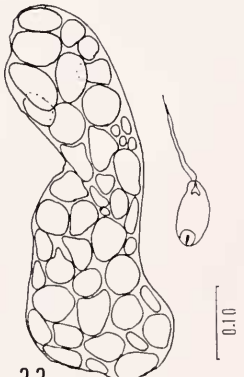

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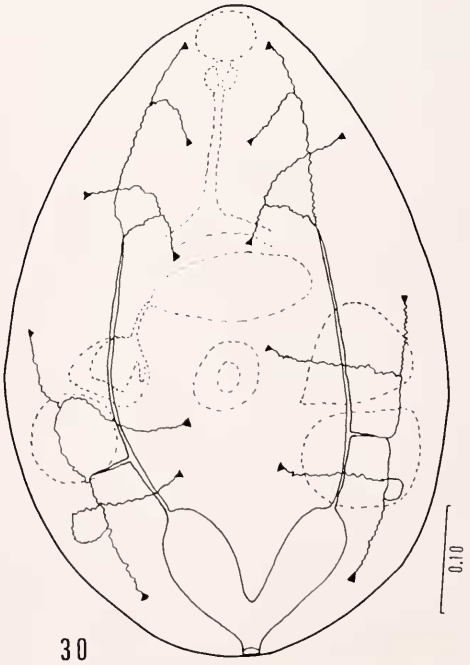

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5 B. The natural definitive host was the raccoon, *Procyon lotor* (L), but possibly other mammals may also serve. The first intermediate host was a snail *Lyrodes parvula* Guilding, 1828 (spined form). The second intermediate host was the blue crab, *Callinectes sapidus* Rathbun, 1896.

Description of the Stages of the Life History of Carneophallus **basodactylophallus** n. sp.

The Adult (Figures 29-30)

SPECIFIC DIAGNOSIS: [based on 10 hot water-killed, unflattened specimens from *Rattus norvegicus albinus* (Sprague-Dawley strain) and *Mus musculus albinus* (ICR strain)]. Microphallidae; *Carneophallus*. Body pyriform, sometimes with posterior notch, 0.415 to 0.455 long by 0.285 to 0.330 wide. Forebody 0.187 to 0.212 long. Integument of anterior 1/2 of body spined. Oral sucker subterminal, 0.050 to 0.062 long by 0.050 to 0.062 wide. Prepharynx almost absent. Pharynx 0.022 to 0.025 long by 0.022 to 0.027 wide. Esophagus extending from pharynx to approximately anterior 1/3 body, 0.087 to 0.110 long. Ceca two, extending posteriorly and obliquely from cecal bifurcation at posterior end of esophagus, 0.125 to 0.152 long. Acetabulum equatorial, mesial, 0.052 to 0.065 long by 0.052 to 0.062 wide. Sucker ratio 1:1.02 to 1.07.

Genital pore sinistral to acetabulum, followed by genital atrium approximately 1.25 times diameter of acetabulum. Testes two, side by side in posterior 1/3 body, edges smooth, oval in outline: dextral testis 0.022 to 0.052 long by 0.065 to 0.095 wide; sinistral testis 0.027 to 0.050 long by 0.050 to 0.095 wide. Vas deferens joining anterior to acetabulum, connecting with dextral posterior margin of seminal vesicle. Seminal vesicle club-shaped, preacetabular, transverse to longitudinal axis of body, tapered to form slender sperm duct surrounded by prostate gland cells at distal tip. Sperm duct connecting with fleshy intra-atrial genital papilla (0.060 to 0.062 long by 0.030 to 0.045 wide) with small posterior basal flap. Ovary between seminal vesicle and dextral testis; oval to oblong in outline, edges lightly lobed, 0.052 to 0.075 long by 0.050 to 0.075 wide. Uterus descending from mesial intertesticular Mehlis' gland to fill posterior body, ascending along body wall to posterior tip of dextral cecum, descending to posterior body, ascending along body wall to posterior tip of sinistral cecum, connecting with slightly enlarged metraterm surrounded by gland cells which in turn connects with genital atrium. Vitellaria composed of 6 to 11 coarse follicles on each side of body, extending from posterior ends of ceca to posterior 1/8 body. Uterine eggs operculate, 16-17.5 microns long by 10-12 microns wide. Excretory vesicle V-shaped; main stem extending anteriorly from mesial excretory pore at posterior end of body, forking at level of testes. Flame cell pattern 2 [(2+2) + (2+2)] = 16.

HOSTS: *Procyon lotor* (L) (type host) [natural host]; *Rattus norvegicus albinus* (Sprague-Dawley strain) and *Mus musculus albinus* (ICR strain), [laboratory hosts].

LOCATION: Small intestine.

TYPE LOCALITY: Mouth of Mississippi River at Pass a Loutre, Louisiana.

HOLOTYPE: U. S. Nat. Mus. Helminthological Coll. No. 70430.

Carneophallus basodactylophallus is distinguished from the other species of the genus by the small posterior flap on the base of the male papilla. The very short prepharynx, even in extended living worms, distinguishes it from all types except *C. turgidus* Leigh, 1958 and *C. choanophallus*.

The Metacercaria

C. basodactylophallus metacercariae were found encysted in the hepatopancreas and muscle tissue of the cephalothorax and all the appendages of the blue crab *Callinectes sapidus* Rathbun, 1896. They are morphologically similar to *C. choanophallus* metacercariae.

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Figures 29-33. *Carneophallus basodactylophallus*. **29.** Adult, dorsal view, drawn from a flattened and stained whole mount. **30.** Excretory system, drawn from observations on living excysted metacercariae and adults. **31.** Frontal section of the adult genital atrium showing the posterior basal flap of the male papilla. **32.** Xiphidiocercaria after emergence from snail. Drawn from living specimen stained with neutral red. **33.** Sporocyst from the spined form of *Lyrodes parvula*.

Laboratory infections of the blue crab took place when cercariae from the spined form of *Lyrodes parvula* were allowed to enter the gill chamber where penetration of the gills took place. Ten of ten (100 per cent) exposed crabs became moderately (50 or more metacercariae) to heavily (100 or more metacercariae) infected versus two of thirty (6.6 per cent) of the controls which were found to be very lightly (1 to 3 metacercariae) infected. Migration of the cercariae in the crab hemocoel was not observed. However, the cercariae wriggled their way to the hepatopancreas and the muscle where encystment took place in thin membranes.

Development of *C. basodactylophallus* in the blue crab seemed to be slower than *C. choanophallus* in shrimp, since after 40 days post-exposure at room temperature the thickened cyst wall of the metacercaria had not yet developed.

Newly formed encysted metacercariae measured 0.050 by 0.035 in diameter. Fully developed metacercariae measured 0.330 to 0.360 long by 0.325 to 0.355 wide. The cyst wall was composed of an inner membrane 0.005 thick and an outer hyaline layer 0.010 to 0.015 thick surrounded by a thick fibrous coat 0.040 to 0.060 thick.

Of the geographical areas checked, the incidence of infection at Pass a Loutre was 85 per cent, and 9.2 per cent at the Willswood and Osgood ponds. Ten crabs taken from the canals of the Bonne Carre Spillway were all negative for *C. basodactylophallus*.

The Cercaria
(Figure 32)

Small "monostome" xiphidiocercaria with the characters of the Ubiquita group. The following dimensions were determined using living cercariae stained with neutral red and Nile blue sulfate. Body 0.080 to 0.120 long by 0.040 to 0.066 wide, ovoid to elongate. Tail 0.060 to 0.110 long by 0.010 to 0.015 wide at base with fine cuticular annulations. Oral sucker 0.025 to 0.030 in diameter. Stylet 0.012 to 0.015 long by 0.004 wide, base rounded, shaft cylindrical then tapered to slightly ventrally directed point. Digestive system not observed. Two thick pairs of penetration gland cell ducts, lateral pair 0.035 to 0.040 long by 0.009 to 0.012 wide, mesial pair 0.030 to 0.035 long by 0.007 to 0.009 wide, opening into anterior portion of oral sucker lateral to stylet, lateral gland cell ducts opening anterior to mesial duct apertures. Cystogenous gland cells not prominently visible. Excretory vesicle U-shaped. Excretory formula 2 [(1 + 1) + (1 + 1)]. Develop in oval or elongate sporocysts containing about 40 to 60 cercariae.

HOST: *Lyrodes parvula* Guilding, 1828 (spined form).

TYPE LOCALITY: Pass a Loutre, Louisiana.

The cercariae of *C. basodactylophallus* closely resemble those of *C. choanophallus*. The notable differences were the shorter stylet with its rounded base, the enlarged penetration gland cell ducts, and the snail host; the spined form of *L. parvula* for *C. basodactylophallus*. The cercariae were incapable of producing metacercariae in *M. ohione*. Conditions for emergence and subsequent activity of the cercariae appear to be similar to *C. choanophallus*.

The Daughter Sporocyst
(Figure 33)

There are probably two sporocyst generations of *C. basodactylophallus* in the spined form of *Lyrodes parvula*, but only the cercariae producing one was observed in naturally infected snails studied in the laboratory. Of 100 spined forms of *L. parvula* from Pass a Loutre, 2 were observed to contain *C. basodactylophallus* cercariae when crushed. The sporocysts were elongate to saccate in shape. They contained from 40 to 60 cercariae, many of which were ready for release. The birth pore was terminal.

The snails examined contained 5 and 30 sporocysts which measured 0.400 to 0.550 long by 0.150 to 0.300 wide. The sporocysts of *C. basodactylophallus* were longer and contained more cercariae than those of *C. choanophallus*.

The Eggs and Miracidium

Morphologically, the eggs of *C. basodactylophallus* could not be distinguished from those of *C. choanophallus*. Measurements were 17-18 microns long by 9-11 microns wide.

No attempts were made to culture the eggs and miracidia were not observed.

Discussion

The erection of the four sub-families within the Microphallidae (Deblock and Tran Van Ky, 1966) provides a realistic and convenient division of the numerous species of microphallids into related groups based on the morphology of the copulatory structures. Following Cable and Kuns (1951) the sub-family Maritreminae (Lal, 1939) Deblock and Tran Van Ky, 1966 would be considered primitive. The sub-family Microphallinae Ward, 1901 could have arisen as one stem from Maritreminae. Evolution in this case is suggested to have proceeded from the primitive forms possessing a cirrus and cirrus sac to forms in which these structures are lost. This loss is thought to be the result of evolutionary modification resulting in the numerous morphological variations of the genital atrium and male papilla presently demonstrated by these forms. The sub-families Gynaecotylinae Guschanskaia, 1952 and Sphairiotreminae Deblock and Tran Van Ky, 1966 may be thought of as having arisen as another stem from a more primitive source or from the Maritreminae as a result of evolutionary modification to a vesiculo-prostatic sac which replaced the cirrus pouch.

The discussion in this paper is concerned with the development of the genera and their species within the sub-family Microphallinae Ward, 1901. Biguet, Deblock and Capron (1958) have continued to follow the reasoning of Cable and Kuns (1951) in their listing of four genera of the Microphallinae (*Leninseniella* Stiles and Hassal, 1901; *Spiculotrema* Belopolskaia, 1949; *Microphallus* Ward, 1901; *Endocotyle* Belopolskaia, 1952) based upon the morphological attributes of the genital atrium and its contained male copulatory papilla. Biguet, Deblock and Capron (1958) considered the genus *Carneophallus* Cable and Kuns, 1951 redundant. They argued that lobation of the male copulatory organ although a morphological fact, is not significant enough to warrant a separate genus. Cable, Connor and Balling (1960) objected to lumping of heterogeneous groups into the single genus *Microphallus,* contending that the adult morphology alone was not a satisfactory basis upon which to determine the taxonomic relationships of the *Carneophallus* group. This writer agrees with Cable, Connor and Balling (1960) and considers the lumping of the many species listed under the genus *Microphallus,* based not upon shape and form of the phallus but upon its comparative dimensions (Biguet, Deblock and Capron, 1958) to be a deviation from the method used to this point. If the development of a thickened ornamented wall of the genital atrium is considered reason enough for the maintenance of the genus *Levinseniella* (Biguet, Deblock and Capron, 1958), then it also seems logical to separate out those species which have developed a large fleshy-lobed male papilla under the genus *Carneophallus.* Except for a few borderline cases (i.e. *Carneophallus muellhaupti* Coil, 1956 which possesses a lightly lobed male papilla), the lobation of the male papilla is strikingly obvious, illustrating further evolutionary modification of this structure as well as convenient division of a relatively large heterogeneous group of species.

This reasoning has prompted this writer to place the two new species described in this paper into the genus *Carneophallus* Cable and Kuns, 1951. Cable and Kuns (1951) distinguished the genus *Carneophallus* as microphallids with a large unornamented thin-walled genital atrium which is almost filled with a lobed fleshy male papilla, one of the lobes being penetrated by the ejaculatory duct. Using this distinction and the original descriptions, the following twelve species are segregated:

C. trilobatus Cable and Kuns, 1951.
C. pseudogonotylus (Chen, 1944) Cable and Kuns, 1951.
C. muellhaupti Coil, 1956.
C. skryabini Caballero, 1958.
C. turgidus Leigh, 1958.
C. chabaudi (Capron, Deblock and Biguet, 1957) Cable, Connor and Balling, 1960.
C. tringae (Capron, Deblock and Biguet, 1957) Cable, Connor and Balling, 1960.
C. canchei (Biguet, Deblock and Capron, 1958) Cable, Connor and Balling, 1960.
C. bilobatus Cable, Connor and Balling, 1960.
C. lactophrysi Siddiqi and Cable, 1960.
C. choanophallus n. sp.
C. basodactylophallus n. sp.

The specific name, *choanophallus,* indicates the collared nature of the phallus. The

term, *basodactylophallus,* describes a phallus possessing a finger or flap at its base. These two characters distinguish these two new species from all the others.

Further support for the validation of the genus *Carneophallus* is obtained from growth studies in definitive hosts. Baer (1943) and Stunkard (1960) pointed out that growth in microphallids takes place in the second intermediate host, which is usually a crustacean. Staged development of *C. choanophallus* in shrimp demonstrated the remarkable increase in body and organ size that takes place at this stage in the microphallid life-cycle. It follows then that no significant growth takes place in the worm during its short-term infection in the intestine of the definitive host. Minor differences in worm dimensions were noted in worms recovered from different hosts. Such interspecific host growth variations have also been studied by Hunter (1952) for *Gynaecotyla adunca.* Hunter's (1952) observations agree with those of *C. choanophallus* in that in normal hosts the worms were smaller than in abnormal hosts. In the case of *C. choanophallus,* these growth variations are minor, but they tend to cast doubt upon the classification system of Biguet, Deblock and Capron (1958) based upon comparative sizes of the male papilla and the ventral sucker rather than morphology for the species of the genus *Microphallus.* The shape and form of the male papilla, which was seen to be characteristically constant in *C. choanophallus* and *C. basodactylophallus* from all hosts observed, appears to be a character of enough stability to warrant the validation of the genus *Carneophallus.*

Cable (1956) and Cable, Connor and Balling (1960) described differences in stylet and cephalic gland morphology of cercariae which they believed to be of taxonomic significance. Cable, Connor and Balling (1960) for this reason maintained that when the life-cycles of members of the genus *Carneophallus* became known, that additional evidence to support the validity of the genus would appear. The cercariae of both *C. choanophallus* and *C. basodactylophallus* possess only two pairs of cephalic glands. Heard (personal communication) also observed *Carneophallus* spp. cercariae with only two pairs of cephalic glands. Except for the cercaria of *Maritrema caridinae* described by Shibue (1951), all microphallid cercariae are described as possessing four pairs of cephalic glands. It cannot be stated that all *Carneophallus* cercariae possess only two pairs of cephalic glands on the basis of the two cercariae seen out of the possible 12 species of these microphallids known. However, this peculiar difference observed in these two species lends further evidence to support the validity of the genus *Carneophallus* Cable and Kuns, 1951.

Experimental infection of *Macrobrachium ohione* and *Palaemonetes pugio* with cercariae of *Carneophallus choanophallus* demonstrated that this microphallid may utilize more than one species of shrimp as the second intermediate host. *Microphallus minus* Ouchi, 1928 in Japan and China is reported to encyst in three species of shrimp (Ouchi, 1928 and Yeh and Wu, 1951), *Macrobrachium nipponensis, Palaemon asperulus* and *P. nipponensis.* Repeated attempts to infect shrimp with *C. basodactylophallus* cercariae and crabs with *C. choanophallus* cercariae failed in this laboratory, thus confirming the taxonomic relationship and second intermediate host specificity of these two very closely related species.

The significance of the second intermediate host specificity demonstrated by microphallids as it might reflect phylogenetic relationships is not clear. A case in point is *Microphallus claviformis* Brandes, 1888 (Syn. *M. limuli* Stunkard, 1951). Deblock and Rosé (1965) discovered the metacercariae of *M. claviformis* in an isopod, *Sphoeroma serratum,* following the invalidation of *M. limuli* by Biguet, Deblock and Capron (1958). Stunkard (1951, 1960) recognized the close resemblance of the excysted metacercariae of *M. limuli* and *M. claviformis,* but because the former were in the chelicerate, horse-shoe crab, *Limulus polyphemus,* which is quite unrelated to the typical arthropod second intermediate host, he was reluctant to regard it a synonym.

The manner in which microphallid cercariae enter the crustacean second intermediate host was first described by Cable and Hunninen (1940). This description is not unlike that observed for *C. choanophallus* in shrimp with one interesting addition. Within 48 hr of penetration and passage into the hemocoel, the *C. choanophallus* cercariae have secreted thin protective mem-

branes which completely surround them, and within which they were seen to continue to migrate toward the shrimp abdominal musculature. The early presence of this membrane suggests that it may serve to protect the cercariae from host antibodies. The presence of the cercariae and encysted metacercariae in the shrimp muscle apparently elicited no significant reaction as shrimp were not refractory to superinfection upon challenge to reinfection. The functions of the cyst wall and the methods by which nutrition and excretion across the wall are accomplished are not well understood at this time, but in view of the energy requirements of the developing larva would form the basis for an exciting study.

Hunter and Vernberg (1953) and Ching (1962 a, b) demonstrated stages in the development and growth of microphallid metacercariae in the second intermediate host. This remarkable growth period in the life-cycle of *C. choanophallus* reached the stage of infectivity to mice when the vitelline glands appeared on about day 30 at 27 C in shrimp. The criteria for infectivity of the definitive host are not known. In this study, the muscular organs: the two oral suckers, the pharynx and the male papilla all appeared at the same time on about day 9. In general the staged development of *C. choanophallus* in shrimp is similar to that described previously for other microphallids.

The necessity of approaching a trematode life history study from the ecological point of view is mentioned in Cable and Hunninen's (1940) elucidation of the life history of *Spelotrema nicolli* as the key to finding the snail which shed cercariae. This was likewise true in the search for the cercaria of *C. choanophallus,* for it was not until a geographical survey led the way to marshes and ponds at the mouth of the Mississippi River where heavy infections of shrimp were found that snails bearing *C. choanophallus* cercariae were discovered. The question as to how shrimp taken from the Mississippi River at Greenville, Mississippi 540 miles from the mouth of the river became infected deserves some comment. It would not be possible for shrimp to migrate such a distance up-stream especially since young 20- to 25-mm shrimp were found infected. Infection must have occurred locally, but since infected snails were never found except at the mouth of the river, one may speculate that another yet-to-be-found snail is responsible, or that since shrimp infections are markedly lower in the regions up-river, the incidence in the snails is correspondingly so low as to make capturing an infected one unlikely. Since the Mississippi River rises and falls in water level several times a year, flooding into and communication with ponds and marshes along the banks, it is reasonable to assume that certain of these ponds not yet visited by this investigator could support populations of snails infected with *C. choanophallus* cercariae.

Rankin (1940) reported that *Gynaecotyla nassicola,* adults of which are harbored by migratory birds, showed a seasonal fluctuation in the snail *Nasa obsoleta,* the highest incidence being in the spring. No report was made on the incidence of infection for the second intermediate host. Studies on the life histories of *Maritrema obstipum* and *Levinseniella amnicolae* by Etges (1953) indicated that the highest incidence in the first intermediate host snail, *Amnicola pilsbryi,* and the second intermediate host, *Asellus communis,* infections were in the late summer and fall. Etges (1953) indicated that the periodicity of these infections was due to the migratory habits of birds which serve as the definitive hosts. In experiments with *C. choanophallus,* time has not permitted an extended survey of the incidence of infection in the first intermediate host, the unspined form of *Lyrodes parvula.* Present collections throughout the winter showed an incidence of 1 to 7 per cent suggesting that these snails may be continuously shedding cercariae whenever conditions permit. Since this study demonstrated that infected shrimp are found throughout the year, the definitive hosts (raccoon and rat) may maintain continuous short-term infections seeding snails at all seasons of the year in the subtropical New Orleans, Louisiana area. It may be that the short-term infections of the definitive host in microphallids serves no other purpose than to disperse the eggs. Sogandares-Bernal and Lumsden (1964) suggested that in short-term infections of *Ascocotyle leighi* Burton, 1956, which encysts in the hearts of certain poeciliid and cyprinodont fishes,

and in microphallids, ovigerous worms may be passed in natural infections, much in the same way the gravid proglottids of certain cestodes are passed from the definitive host. They pointed out that the short-term infection of the definitive host is probably the result of an evolutionary sequence which has allowed for the worms to continue with their biological function of reproduction without harming the host. The usual long-term infection with these parasites could lead to the death of the definitive host if the minute eggs were to enter the circulatory system with resultant complications (Africa, Garcia and de Leon, 1935a, b, 1936, 1937).

The 15-month study of *M. ohione* infected with *C. choanophallus* demonstrated two seasonal upsurges in incidence and intensity of infection with the heaviest infection occurring in the late summer and early fall. These two upsurges in *M. ohione* infection were seen to follow two periods of recruitment of young shrimp into the samples suggesting that the infection in *M. ohione* occurred mainly in young shrimp twice a season. The fact that juvenile shrimp in endemic areas are usually found in shallow still water containing vegetation upon which the unspined form of *Lyrodes parvula* is normally found substantiates this point of view. This does not rule out the possibility that infection of older shrimp, which are found in deeper swift moving water may take place continuously at a low level throughout the year with activity declining during the cold winter months.

Acknowledgments

This study was done under the direction of Dr. F. Sogandares-Bernal. Acknowledgments are extended to Dr. Norman C. Negus, for advice on the ecological portions of this work, to Dr. John C. Hitt, for advice on computerization of the data and its analysis, to Dr. Harold W. Harry, for identification of the snail hosts, to Dr. Emile A. Malek, for aid in the collection of snails, to Dr. W. A. Eggler, for identification of aquatic plants, and to Dr. A. E. Smalley, for confirming the shrimp identifications.

I am especially indebted to Dr. T. B. Ford and Mr. Allan Ensminger, Louisiana Wildlife and Fisheries Commission, for use of facilities and other aid.

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March 24, 1969